THE ACTION OF ETHER AND CHLOROFORM ON THE NEURONS OF RABBITS AND DOGS.

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THE following investigation was undertaken to determine whether chloroform and ether produce any transient or permanent changes in the cortical and spinal neurons.

In the first series of experiments, rabbits were used. The animals were handled as gently as possible in order to avoid abdominal congestion and consequent cerebral anæmia. The drug was administered by a tube in the trachea. In the case of rabbit No. 1, the animal was killed by excess of ether; in the remaining cases the organs (brain and cord) were removed during the anæsthesia, and this operation was the obvious cause of death.

Fifteen minutes before each animal was killed, administration of the anæsthetic was suspended, and the time of return of the conjunctival reflex noted. The administration of the anæsthetic was then resumed until the animal was killed. The following table gives (1) the time during which anæsthesia was kept up, (2) the time taken for the return of the conjunctival reflex; this may be regarded as a rough indication of the functional depression in the neurons concerned in the reflex, and so also as an index of the depression in all the nerve-cells implicated by the anæsthesia.

	Anæsthetic		Time of return of reflex
Rabbit 1.	Ether	35 minutes	2 minutes
,, 2.	,,	1 hour	$2\frac{3}{4}$,,
,, 3.	,,	2 hours	4 ,,
,, 4.	,,	4 ,,	$5\frac{1}{2}$,,
,, 5.	,,	6 ,,	$6\frac{3}{4}$,,
,, 6.	Chloroform	30 minutes	2 ,,
,, 7.	,,	1 hour	$2\frac{1}{2}$,,
,, 8.	,,	2 hours	4 ,,
,, 9.	,,	4 ,,	6 ,,
,, 10.	,,	6 ,,	$7\frac{1}{4}$,,

In a second series of experiments which were performed on dogs, the additional precaution was taken to keep up the body temperature by placing the animal on a hot water bed, and covering it with cotton wool. I did this because it had been suggested by Dr Head at the International Physiological Congress, at Cambridge, when I made a preliminary communication on my experiments with rabbits, that some of the changes I there described might be due to lowering of temperature. The following table gives the details of length of anæsthesia, time of return of conjunctival reflex, and body temperature.

	Anæsthetic		Time of return of reflex	Maximum and minimum temp.
Dog 1.	Ether	30 minutes	½ minute	101—101°
„ 2.	„	1 hour	1½ minutes	101—99·2°
„ 3.	„	2 hours	2½ „	101·5—98·75°
„ 4.	„	4 „	3½ „	101—98°
„ 5.	„	6 „	5 „	101—98·25°
„ 6.	Chloroform	30 minutes	½ „	101·5—101·5°
„ 7.	„	1 hour	1½ „	100·5—100°
„ 8.	„	2 hours	2¾ „	101—99·8°
„ 9.	„	4 „	4 „	101—98·5°
„ 10.	„	6 „	5½ „	101—97·8°

I did not measure the amount of anæsthetic actually given in the various experiments; some preliminary attempts to do this did not yield satisfactory results.

The organs removed from the animals (brain, and cervical region of the spinal cord) were divided into two parts longitudinally; one half was placed in absolute alcohol, the other in Müller's fluid. The tissues fixed in alcohol were worked out by Nissl's methylene blue method, by Weigert's hæmatoxylin and by the hæmatoxylin-eosin stains. Those fixed in Müller's fluid were investigated by Berkeley's modification of Golgi's method in the case of the rabbits, and by Cox's method in the case of the dogs.

The following paragraphs give the result of the microscopic examination in each animal. The sections from the tissues fixed in alcohol were cut to a uniform thickness of 15μ, and any description given is from observation of those cells that were cut fairly through their median planes.

Rabbit 1. Ether for half-an-hour.

(*a*) *Cerebrum.* All the cortical cells are seen by the aniline dyes to have undergone a change. This is most marked in the layer analogous to the

pyramidal layer in man. Nissl's bodies have almost entirely disappeared from the apical and basal dendrons, and many are absent from the cell bodies, leaving clear untinted spaces. Those yet *in situ* are pale, granular looking and reduced in size; the hyaloplasm between the Nissl's bodies is unstained as in normal cells. There is no breaking down of the chromophilic granules into dust-like particles and diffuse staining of the hyaloplasm such as occurs in degenerating cells. The chromatic substance seems to have merely undergone some change so that it no longer reacts in the usual way to methylene blue. The term *rarefaction* may be employed for the appearances observed.

By the silver-chrome method many processes stain as they do in normal tissue, but in the majority of cases the most distant visible part of the apical dendron and its primary and secondary branchlets, show small moniliform swellings (see fig. 1 in text). The cells from which they originate appear normal by this method.

Fig. 1. Cortical cell of rabbit; moniliform enlargement of the distal portions of the apical dendron is seen. Ether anæsthesia for half-an-hour.

The basal dendrons are but rarely affected.

(*b*) *Cerebellum.* No change.

(*c*) *Spinal cord.* No change was seen in any cells by the methylene blue process. In this, as in the cords of the remaining rabbits, I was not successful in obtaining satisfactory preparations by the silver method.

(*d*) *Vascular conditions.* In brain and cord and their membranes, there is capillary anæmia and venous engorgement. The peri-capillary spaces

are widened and contain a scant number of leucocytes. In the cerebellum the capillary anæmia is not so marked.

Rabbit 2. Ether for one hour.

(*a*) *Cerebrum.* The 'rarefaction' just described is now more general throughout the cell layers, but the pyramidal cells are especially involved (see Plate 1, fig. 1). A few of these have the appearance of mere skeletons, but their outlines are firm, and their nuclei and nucleoli sound. Occasionally in these 'skeleton cells' a faintly stained network with irregular meshes is seen. Glia cells appear swollen, but are not increased in number.

By the silver method, only a few of the apical processes of the pyramidal cells are normal. The majority show the moniliform enlargements noted in the first rabbit. The change is, however, more marked; the enlargements are more numerous and extend farther down the main stem. A large number of basal dendrons are also implicated. Almost all the dendrons arising from the median layer of cells are in some degree moniliform.

(*b*) *Spinal cord.* There is 'rarefaction' in a small number of anterior cornual cells, but no change in the cells of the posterior horn.

(*c*) *Vascular conditions.* The same as in the first case.

Rabbit 3. Ether for two hours.

(*a*) *Cerebrum.* A process of restitution seems to have set in during the second hour. The dendrons are still totally denuded of Nissl's bodies, but the cells themselves appear to have become rehabilitated to a noticeable degree. A few, however, show as great a 'rarefaction' as in previous cases. All the cortical glia cells are swollen and pale; they appear to be augmented in number and tend to aggregate in the vicinity of the rarefied cells.

By the silver method, nearly every dendron is seen to be moniliform; and the swellings have increased in size, notwithstanding the apparent restitution in the cell bodies. The cells from which the affected dendrons spring look healthy, though probably those which are most implicated spring from the cells which by the methylene blue process would have been shown to be most rarefied.

(*b*) *Cerebellum.* No change.

(*c*) *Spinal cord.* The cells are not more deeply implicated than at the end of one hour of etherisation.

(*d*) *Vascular conditions.* The same as before except that the number of leucocytes in the peri-vascular spaces has slightly increased. There are no observable lesions in the vessel walls.

Regarding the restitution which occurred in this case, I was at first inclined to the opinion that the process was *ante-mortem* and represented a successful effort on the part of the cells to throw off the effects of the drug

My subsequent work did not confirm this view. I now believe the effect occurred after the removal of the organs from the body; there was in this case an unavoidable delay of five or six minutes in transferring the organs to the fixing solutions. During this pause, there was doubtless an escape of ether from the blood and lymph, and consequently from the 'surviving' cell bodies.

Rabbit 4. Ether for four hours.

(*a*) *Cerebrum.* The greater number of cells are again rarefied, and many are reduced to mere skeletons. The layers of cells above and below the pyramidal layer are slightly rarefied. The pyramidal cells are markedly rarefied, and a certain number of these show a more marked change than had been noticed in the previous animals; the margins of these cells are disintegrated, their nuclei are eccentric, swollen and granular, the nucleoli are enlarged and irregularly stained. One would hesitate to describe such cells as degenerated, for there is nothing to indicate that the change is a permanent one, or that any cells have completely broken down. The glia cells are indubitably augmented in number and noticeably turgid; they with many leucocytes cluster about and in not rare cases actually penetrate within the most profoundly affected cells.

By the silver stain, all the apical dendrons are seen to be moniliform. In numerous instances the swellings are larger than any observed in previous cases. The lower portions of the dendrons are also more frequently affected. No rupture is apparent in any dendrons. The method reveals no change in the cell bodies.

(*b*) *Cerebellum.* A few of the cells of Purkinje are decidedly rarefied, but the vast majority are normal.

(*c*) *Spinal cord.* The posterior cornual cells are for the first time noticeably rarefied, though the majority are still normal. The anterior horn cells are rarefied (Plate 1, fig. 2), and a small number of these show the pseudo-degeneration just described in the cortical cells. The glia cells show the same change and behaviour as in the cortex. Leucocytes are numerous in the tissue, and mixed with swollen glia cells in the peri-cellular and vascular spaces.

(*d*) *Vascular conditions.* These are practically the same as in previous cases, except that more leucocytes are seen in the peri-capillary spaces, and also a few swollen pale glia cells. There is no appearance of stasis. The nuclei of the capillaries slightly bulge, and stain more deeply than normal.

Rabbit 5. Ether for six hours.

(*a*) *Cerebrum.* Here the same general condition obtains as in the last case, but the number of skeleton cells (Plate 1, fig. 3) is greater, and those

in which the disintegrative change has gone further are also more numerous. Small masses of débris with occasionally a pale swollen nucleus can be seen in any section; these are encompassed by swollen glia cells, and leucocytes; in other instances a mass of enlarged, pale glia cells and leucocytes filled with products of degeneration, and scattered débris amongst them are seen. These possibly mark destroyed cells (Plate 1, fig. 4). Compared with the one hour case, the number of glia cells is enormous.

By the silver method, practically the same results are noted as in the four hour animal, though occasionally the bulbous enlargements are larger (fig. 2 in text).

Fig. 2. Cortical cell of rabbit. The moniliform enlargements have increased in size and extent after six hours' etherisation.

In the cells lying outside the pyramidal layer fine moniliform swellings are not infrequently seen in the most distal parts of their dendrons.

(*b*) *Cerebellum.* A larger number of cells of Purkinje are rarefied than in the last case, and some are reduced to mere skeletons.

(*c*) *Spinal cord.* Only a few anterior horn cells remain normal. A small number show extreme change (Plate 1, fig. 5), and quite two-thirds of the remainder are markedly rarefied. Skeleton cells are more numerous than in the last case, and more of the posterior horn cells, especially the larger ones, are affected. The glia cells appear as in the last case.

(*d*) *Vascular conditions.* These are as before. There are many more leucocytes in the peri-capillary spaces and mingled with them swollen glia cells containing granular material. The latter feature is not so remarkable in the cord as in the cortex.

Rabbits 6, 7, 8, 9, 10. Chloroform for ½, 1, 2, 4, 6 hours respectively.

The changes in the chloroformed animals differ so little in kind or even in degree from those anæsthetised by ether, that it is only necessary for me to note the few differences that were observable.

In the two first of the series (chloroform for ½ and 1 hour) the cellular rarefaction is slightly greater than in the corresponding ether cases.

At the end of two hours (Rabbit 8) the rarefaction is rather more obvious, and there was no diminution of the degree of rarefaction as in the corresponding ether case (Rabbit 3).

After four and six hours the changes noted in the corresponding ether cases are present to a more marked degree, in cerebrum and cerebellum, whereas the changes in the spinal cord are about the same whichever anæsthetic is used.

By the silver method, moniliform swelling of the dendrons is present from first to last, and increases in degree *pari passu* with the anæsthesia.

The vascular conditions are the same as in the ether cases, except that the veins were like the capillaries found to be almost empty and contracted.

Dog 1. Ether for half-an-hour.

No change is observable in any cells by either method. Venous engorgement and capillary anæmia are present as in the rabbits.

Dog 2. Ether for 1 hour.

No change is observable in any cells by Cox's method. By the methylene blue method no change is seen in the cells of cerebellum and cord, but in the brain the Nissl's bodies in many of the pyramidal cells are pale and granular in appearance, and the dendrons of these cells are denuded of Nissl's bodies. The same vascular conditions are present as in the first dog.

Dog 3. Ether for two hours.

Practically the same appearances are present as in dog 2. More pyramidal cells are, however, affected, but moniliform enlargement of the dendrons is not seen. The glia cells are slightly swollen, but stain well. Spinal and cerebellar cells are still normal.

Dog 4. Ether for four hours.

(*a*) *Cerebrum.* The large pyramidal cells are decidedly rarefied (Plate 1, fig. 6). The main apical processes are usually denuded of Nissl's bodies; the basal dendrons occasionally show the same change. The edges of such cells are ragged. A few skeleton cells are seen. The glia cells are more swollen, but show no augmentation. By Cox's method the extreme tips of the apical processes are moniliform, but the swellings are small (fig. 3 in text). The basal dendrons are still normal.

(*b*) *Cerebellum.* A small number of the cells of Purkinje are rarefied.

(*c*) *Spinal cord.* Very occasional rarefied cells are seen in the anterior horn, but the majority of these cells, and all the cells in the posterior horn, are normal.

Fig. 3.

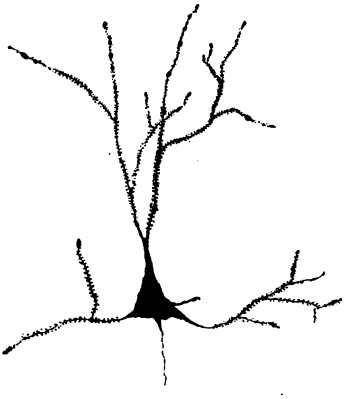


Fig. 4.

Fig. 3. First detected appearance of moniliform change in apical dendron of dog's pyramidal cell after four hours' etherisation.

Fig. 4. Moniliform swellings in dog's pyramidal cell after six hours' etherisation.

(*d*) *Vascular conditions.* Capillary anæmia and constriction are present as in the previous cases. The nuclei of the capillaries bulge and stain rather more deeply than normal.

Dog 5. Ether for six hours.

The condition seen is simply an exaggeration of that in the previous case. The change in the cortical cells has not advanced beyond 'rarefaction' but the glia cells are more numerous and cluster about the markedly affected cells. The next figure in the text (fig. 4) shows the increase in moniliform change. The cells of cerebellum and spinal cord have about the same condition as in the four hour case.

Dogs 6, 7, 8, 9, 10. Chloroform for $\frac{1}{2}$, 1, 2, 4, 6 hours respectively.

The changes observed in these animals are of a precisely similar character to those just described in the case of ether. At the end of four hours the rarefaction is slightly more general and more advanced. Plate 1, fig. 7, shows one of the pyramidal cells in the skeleton stage at the end of six hours' anæsthesia. With regard to the vascular condition, there is less marked capillary anæmia, and the veins are less congested. This agrees with what was observed in rabbits.

It should be mentioned that the anæsthetics were administered to dogs by the mouth, except in the six hour cases (dogs 5 and 10), where a tracheal tube was used.

Conclusions.

The following conclusions can be drawn from the appearances described:

1. In rabbits both ether and chloroform anæsthesia cause certain changes in the nerve-cells of both brain and spinal cord. These are slight at first, but become more pronounced as the anæsthesia is continued. By the methylene blue method, the principal change is that which I have described as 'rarefaction'; in the advanced cases, I have introduced the term 'skeleton cell,' and in the most marked cases of all, a 'pseudo-degenerative' change has set in.

2. In rabbits also there is an early and constant moniliform enlargement of the tips and stems of the chief dendritic extensions of many pyramidal cells; these enlargements grow in size and spread along the dendrons towards the cell body as the anæsthesia is continued. Though it was of course impossible to examine the same cells by both the methylene blue and the silver processes, I brought under observation the cells of nearly identical parts of the two hemispheres, and I consider it justifiable to assume that the moniliform dendrons spring from the cells that show rarefaction.

3. In dogs, there are practically no changes up to two hours, but between that time and four hours changes occur in the nerve-cells similar in kind to though less in degree from those observed in rabbits; these changes become more marked as the anæsthesia is continued.

4. Corresponding to this, the nerve-cells of dogs show no moniliform enlargement of their dendrons up to two hours of anæsthesia, but after this point and presumably at the time that changes occur in the cell-bodies, a few dendrons show moniliform change and this becomes more pronounced as the anæsthesia is continued.

I feel inclined to the view that the lesser degree of the affection in dogs is due not so much to my care in keeping up the body temperature of these animals, but rather to the fact that the neurons of dogs have more inherent power of resistance to the drugs than those of rabbits. In dogs the time of the return of the conjunctival reflex is shorter than in the corresponding rabbits, so the degree of narcosis is less, and it is a common experience that rabbits succumb more readily to anæsthetics than dogs.

I also regard the changes observed in the cells and their processes to be due directly to the influence of the anæsthetics, and not indirectly due to the capillary anæmia which is produced. Mott[1] has produced changes somewhat resembling those I have described by suddenly cutting off the arterial supply to the brain, but there is an obvious difference between such an experiment, and the capillary anæmia I have described. I should regard the anæmia in my experiments as a 'conservative process,' secondary to a diminution in the activity of the nerve centres.

The venous congestion again appears to be a secondary phenomenon, and cannot be regarded as the cause of the changes in the nerve-cells. I think this is quite clear when one compares the effects of chloroform with those of ether; for under chloroform the effect on the nerve-cells is greater, whereas the amount of venous congestion is less.

Ether and chloroform are generally stated to circulate in the blood as such and no bio-chemical change in the blood has hitherto been described as a result of their administration. I am therefore forced to the conclusion that the neuronal changes are bio-chemical in nature, and are produced by the anæsthetic that reaches them *via* the blood stream.

Nissl's theory is that healthy nerve-cells fixed and stained in a constant manner will appear the same under constant optical conditions, and the appearance seen is the equivalent of such healthy nerve-cells during life. It follows that if nerve-cells under the same constant conditions present a difference from the equivalent or symbol of healthy cells, the difference is the measure of some change that occurred during life. To decide how such changes are produced by anæsthetics is a difficulty; there is nothing to suggest that chloroform or ether could cause these changes mechanically, and the supposition that they act chemically is extremely probable. It is possible for instance that the anæsthetic forms a compound with the Nissl bodies and thus prevents them from reacting to stains in a normal way; the degree of this change is proportional to the length of time to which they are exposed to the action of the drugs.

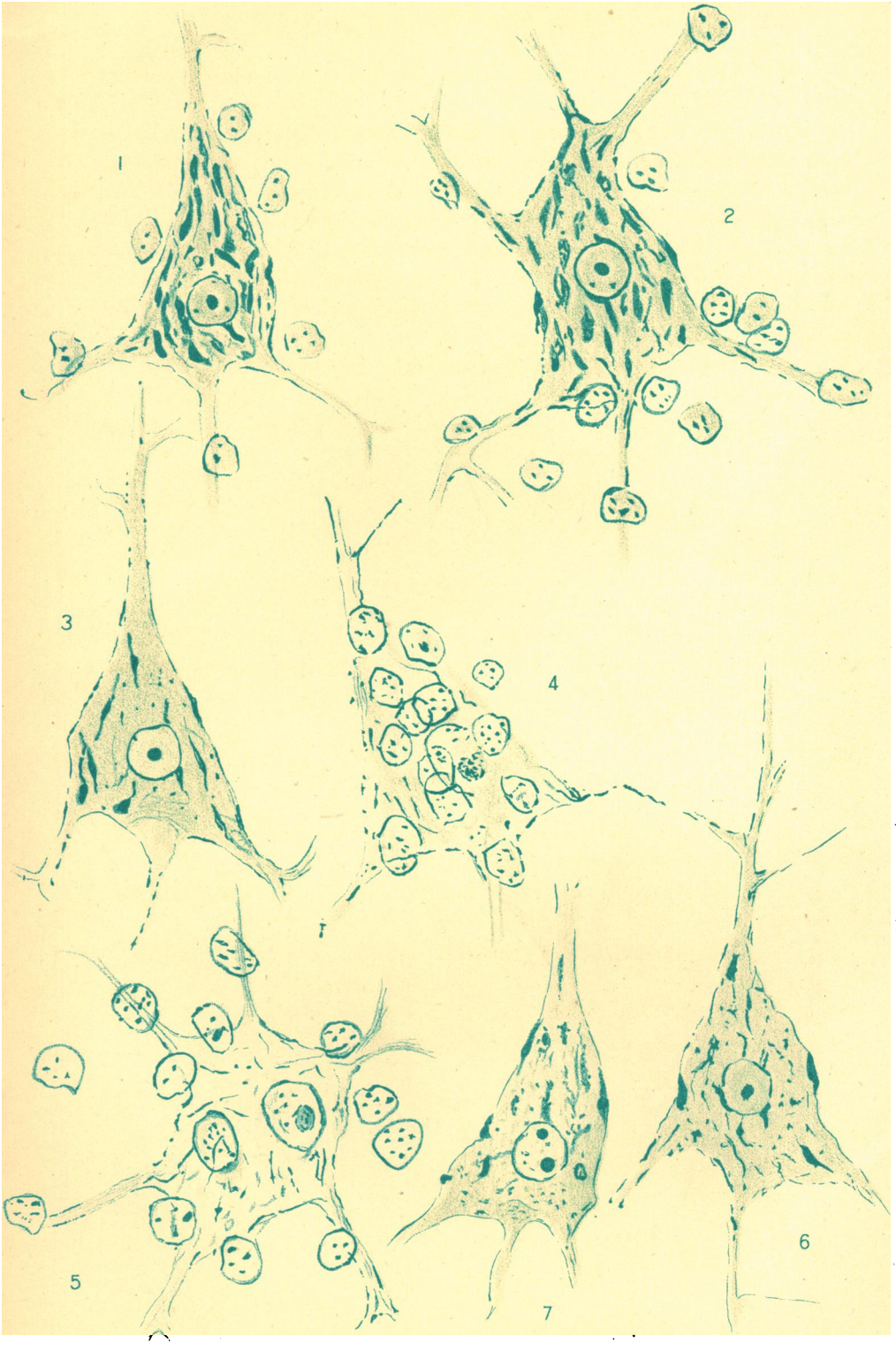
It is generally admitted that Nissl's bodies, forming as they do so large a component of the cell body, are in some way nutritional and energy-producing. Changes in them must necessarily be followed by some modification visible or invisible of the other structures in the cell

[1] *Lancet*, June 30th, 1900.

body and its branches; this is probably the explanation of the moniliform swellings of the dendrons. A large experience in the histological methods employed in this research has convinced me that the varicosities are not due to *post-mortem* changes, and I have seen nothing in my work which confirms a widely spread belief that the enlargements are primarily the bio-physical basis of loss or modification of consciousness. The change is essentially nutritional or bio-chemical, affecting primarily the cell body and secondarily the processes that spring from it. The puffing out of the dendrons occurs first at the points furthest removed from the nutritional centre in the cell, and it is not until the dose of the drug is increased that the moniliform enlargements grow in size, become more numerous and encroach upon the gradually weakening dendritic stem. The phenomenon, whether it is due to simple hydration at the swollen parts, or to a more profound chemical change, appears to be quite analogous to, though less in degree than the degenerative changes that occur when the neuronal body atrophies; the first changes are observed at the most distal portions of the nerve fibre that originates from the cell body, and one may even compare the moniliform enlargements to the increase and swelling of the protoplasm around the nuclei of the sheath of Schwann that occurs in Wallerian degeneration. The bio-physical theory rests principally on preparations made by the use of one or other modification of Golgi's method. Important light is thrown on the question when the aniline stains are employed as well, as by these alone can changes in the cell body be detected.

Lugaro denies the existence of dendritic varicosities as the result of chloroform anæsthesia, and has substituted for the bio-physical theory of Demoor and other writers, another which appears to be still more difficult of proof. According to Lugaro varicosity of dendrons and retraction of neurons is the condition on which consciousness depends, and loss of consciousness depends on the absence of varicosities and retraction, and therefore on the contact of neurons.

If Lugaro's dogs had been anæsthetised for a sufficiently long time, he would doubtless have found moniliform swellings; he makes no mention of the time during which anæsthesia was kept up, and I must conclude that the length of time must have been insufficient. His method of fixing the tissues I regard as a point of secondary importance. He injected Cox's fluid by the carotid, and thus the tissues were fixed *in situ*. If the varicosities I observed were the result of *post-mortem* changes due to my placing the pieces of brain in Cox's fluid in the ordinary way, the varicosities would have appeared in all cases, but



Edwin Wilson, Cambridge.

they did not appear until anæsthesia has been prolonged for four hours.

Whether the facts I have described as occurring in dogs and rabbits, occur also in the human subject under the influence of anæsthetics, it is obviously impossible to say. But I do not consider that there is any analogy between the changes I have described, and those bio-chemical anabolic and katabolic changes that occur in daily life and mark our sleeping and waking hours. I regard the action of narcotics such as those I have employed as pathological, not very intensely pathological it is true, but still something which is remote from physiological processes. In sleep there is probably an opportunity for the constituents of the nerve-cells to undergo anabolic changes, whereas in the unconsciousness produced by anæsthetics, the process appears to be associated with an exhaustion of them.

The expenses of this work were defrayed by a grant made to me as John Lucas Walker Exhibitioner of the University of Cambridge. The experiments themselves have been performed partly in the University of Heidelberg, partly in the Pathological Laboratory at Cambridge, and partly in the Physiological Laboratory at King's College, London. A good deal of the microscopic examination of the tissues was carried out in the London County Asylums Laboratory, Claybury, and a few experiments have been completed since my arrival in the Malay States.

EXPLANATION OF PLATE I.

Fig. 1. Moderate 'rarefaction' of large pyramidal cell of rabbit after one hour's administration of ether.

Fig. 2. Rarefied anterior cornual cell of rabbit after four hours' etherisation.

Fig. 3. 'Skeleton' pyramidal cell of rabbit after six hours' etherisation.

Fig. 4. Pyramidal cell of rabbit after six hours' etherisation in which the change has advanced beyond the skeleton stage.

Fig. 5. Anterior cornual cell of rabbit after six hours' etherisation showing the same advanced change.

Fig. 6. Rarefied pyramidal cell of dog's cortex after four hours' etherisation.

Fig. 7. 'Skeleton' pyramidal cell of dog's cortex at the end of six hours' chloroform anæsthesia.